Winter distributions of *Dinophysis* populations: do they help predict the onset of the bloom?

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Abstract

Blooms of diarrhetic shellfish toxin (DST) producers of the genus *Dinophysis* (*D. acuminata, D. acuta*) pose the main threat to the sustainable exploitation of cultivated mussels and other bivalves on the Atlantic coasts of Europe. *Dinophysis* species do not rely on cysts as a seeding strategy. Detection and evaluation of holoplanktonic populations surviving after bloom decline may be the key to predict the initiation of next year's bloom. Three cruises were carried out on the NW Iberian shelf in February 2013 (DINVER 2013), January 2006 (DINVER 2006) and May-June 1993 (MORENA 93) to explore winter (*D. acuminata*) and pre-bloom (*D. acuta*) distributions of harmful microalgal species. Sampling protocols were adapted to be able to detect extremely low densities (1-5 cells l^{-1}) of *Dinophysis* species. Potential inoculum populations in retention areas, as previously described for other species in upwelling regions, were not found on these cruises. Here we explore retrospectively data from these cruises, identify hydrodynamic patterns, and accompanying microplanktonic communities, in an attempt to untangle a crucial question in *Dinophysis* population dynamics: how to predict the initiation of the *Dinophysis* growth season.

Keywords: Dinophysis distribution, overwintering cells, *Dinophysis acuminata*, *Dinophysis acuta*

Introduction

Dinoflagellate species of *Dinophysis* produce lipophilic shellfish toxins (DSP toxins and pectenotoxins) and pose a worldwide threat to sustainable exploitation of shellfish resources (Reguera *et al.* 2014). Endemic blooms of *Dinophysis acuminata* and *D. acuta* cause lengthy shellfish harvesting closures in aquaculture sites on the European Atlantic coast. In the Galician Rías (NW Iberia), *D. acuminata* can be associated with DSP outbreaks within the whole upwelling season (March-October), but *D. acuta* is very seasonal and outbreaks caused by this species usually occur during the autumn upwelling transition (Escalera *et al*. 2006, 2010). Considerable knowledge has been gained on the population dynamics of *Dinophysis* species, but the causes of their interannual variability and the origin of the inoculum remain poorly understood (Reguera *et al*. 2012). In sexual cyst-forming species with mandatory resting periods, yearly recruitment of new cysts to the top sediment layer can be tracked and used in prediction models (Anderson *et al*. 2014). *Dinophysis* species have complex polymorphic life cycles including sexual processes, but the existence of sexual cysts has not been proven either in field populations or in laboratory cultures (Escalera and Reguera 2008). An alternative procedure is to explore the relationship between overwintering mobile cells acting as "pelagic seed banks" (Smayda 2002; Smayda and Trainer 2010) and the initiation of the species growth season. Weekly monitoring has proven to be insufficient to detect rapid changes in numbers due to wind-direction reversals and subsequent DSP outbreaks (Whyte *et al.* 2014). This stresses the need for modeling/operational oceanography approaches.

One objective of the EU project ASIMUTH was the "*Identification of key past events which will be re-analysed and used for training the modeling system*" (www.asimuth.eu). In this framework, we revisited results from three mesoscale cruises carried out on the NW Iberian shelf. Our main question was whether pre-bloom/overwintering distributions of *Dinophysis* species on the shelf are useful to predict the initiation of *Dinophysis* growth season in the Galician Rias Bajas.

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Material and Methods

CTD casts and water samples (Niskin bottles) for phytoplankton analyses (Utermöhl method, specimens from the whole chamber at 100X) were collected on three cruises on the NW Iberian shelf. Ekman transport was estimated from model data of the US Navy's Fleet Numerical Meteorology and Oceanography Centre (FNMOC) derived from sea level pressure on a grid of approximately 1°x1° centred at 43°N 11°W, a representative location for the study area.

MORENA 93 (May 5-31, 1993): carried out on board RV *Cornide de Saavedra*, sampled 13 transects (92 stations) perpendicular to the coast between Cape Finisterre (43ºN) and Mondego River (40ºN) at a time of the year when *D. acuta* (target species) is usually below detection levels in the Galician Rías Bajas. Lugol-fixed water samples (250 ml), collected at several depths, were left to settle in glass measuring cylinders over 2d before siphoning out to a final volume of 50ml, sedimentation and counting (detection level, 4 cells l^{-1}).

DINVER 2006 (Jan 31 - Feb 2, 2006) was carried out on the Galician shelf and outer reaches of the Rías Bajas on board RV *Mytilus* in early winter, when *D. acuminata* cells are hardly detectable in the Rías. Stations (47) were chosen after real time simulations of water velocities with the MOHID model (Carracedo *et al*. 2006) so as to include 2 transects and different points located within anticyclonic eddies suspected to act as retention areas for HAB species. Water samples (2.5 l) were passed through a PVC cylinder with a 20-µm sieve, to a final volume (to be measured) of around 50 ml, and 25 ml were sedimented for cell counts (detection level ~1 cell l^{-1}).

DINVER 2013 (Feb 27 - March 1, 2013), on board RV *Ramón Margalef* in mid winter, surveyed 7 transects (45 stations) distributed in a fixed grid. Samples (1-2.5 l) were filtered through nytex filters (20-µm, 47 mm Ø) that were resuspended in 50 ml of filtered seawater with Lugol's solution before sedimentation of 10 ml for counting (detection level, 2-5 cells l^{-1}).

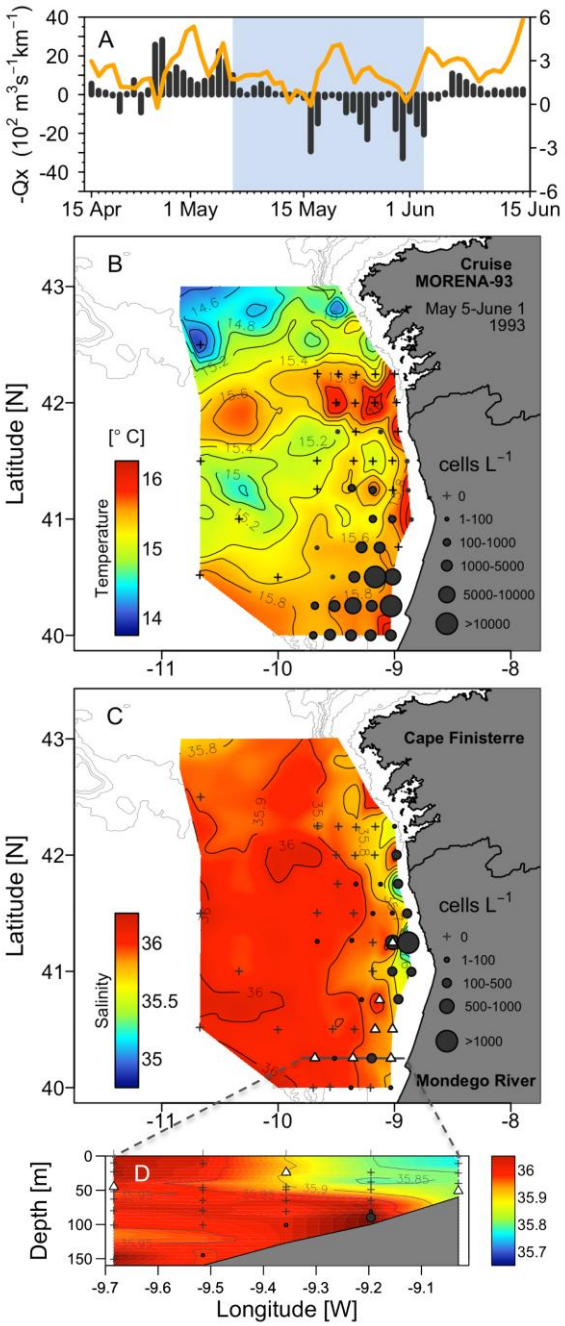

Fig. 1. MORENA 93. A: Daily Ekman transport (orange line shows the 1985-2013 mean; shaded area, the cruise period; B: Temperature (5m) and *G. catenatum* cell maxima; C: Salinity (5m) and *D. acuminata* cell maxima distribution (white triangles represent *D. acuta* ≤8 cell·l^{-1}) and D: Vertical distribution of salinity, *D. acuminata* cells and *D. acuta* presence.

Results and Discussion

MORENA 93 started under upwelling conditions followed by relaxation until mid May. From then, until after the cruise, strong and highly variable SW winds caused prolonged downwelling conditions, most unusual at this time of the year (Fig. 1A), and surfacing of the Iberian Poleward Current (IPC, S>35.9). The latter formed a strong tongue-shaped density gradient close to the

Portuguese coast (40-42ºN), but nearer to the shelf break off the Galician Rías (42-43ºN) (Fig. 1B).
There was a strong latitudinal heterogeneity in the micro-phytoplankton distribution (data not shown) with two well differentiated parts: a northern half with dominance of diatoms and a southern half, dominated by dinoflagellates, including a bloom of the PSP agent *Gymnodinium catenatum*, with cell maxima in the pycnocline at about 50m (Fig 1B). Moderate to low densities of *D. acuminata*, which had been reported by the Galician monitoring programme since February, were found near the coast in the whole area. *D. acuta* at extremely low densities (4-8 cell l^{-1}) was detected at 7 stations in the southern half. A vertical section showed that these cells were in the top 50-m layer, whereas *D. acuminata* cells were near the seabed (Fig. 1D).
DINVER 2006. The objective of this cruise was to look for overwintering populations of *D. acuminata*. Conditions in the outer reaches of the Galician Rías showed temperature inversions, typical for the area in mid winter; the upwelling season had not started. Micro-phytoplankton was very scarce, but with a good contribution of large dinoflagellates (*Ceratium azoricum*, *C. candelabrum*, *C. pentagonum)* that occur this time of the year associated with the Iberian Poleward Current. In addition, there were scattered cells of *G. catenatum* and *D. acuta* that were remains of intense blooms of these species in November 2005 (Pizarro *et al*. 2008) that caused harvesting closures due to DSP (until the end of January) and PSP (until May), toxins above regulatory levels until mid-spring of the next year (ICES 2006). A few isolated cells of *D. acuminata* were detected at only 2 stations in the outer reaches of the northern margins of Ría de Muros and Ria de Pontevedra (Fig. 2).
DINVER 2013: The upwelling season had started, and large diatoms (e.g. *Ditylum brightwellii*) and *Chaetoceros* spp. were dominant in the photic zone throughout the Galician shelf. *D. acuminata*, which had been detected in the Rías Baixas all through the winter, was found in low numbers (< 40 cell l^{-1}) everywhere except at some stations in the northernmost transects. Densities were slightly higher in the mouths of Ría de Vigo and Ría de Pontevedra due to advection.
The three cruises were carried out coinciding with anomalous conditions for their time of the year, but we examined retrospectively the information we can draw from each one concerning prediction

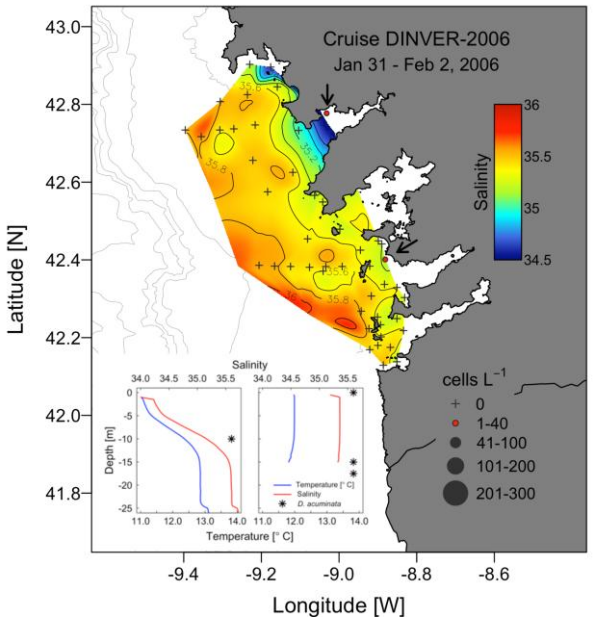

Fig. 2. Surface salinity distribution during DINVER 2006 cruise. Vertical profiles correspond to the 2 stations (red dots and black arrows) where scattered cells of *D. acuminata* were found.

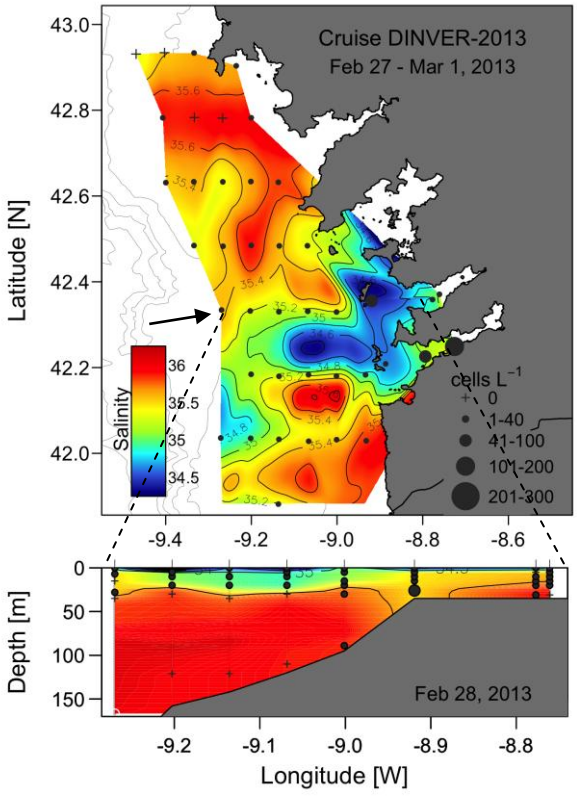

Fig. 3. Salinity (5m) and *D. acuminata* distribution during DINVER-13, and a vertical profile of a transect from Ría de Pontevedra to the shelf (marked with an arrow).

of the initiation of forthcoming blooms of *Dinophysis* species.

In the case of MORENA 93, conditions in May recalled those typical in September-October when the upwelling season is finished. But still *D. acuta*, a species whose autumn blooms in the Galician Rías usually precede (a few days difference) those of *G. catenatum*, was detected only in the southern half of the survey area. This observation confirms this species is a seasonal (late summer-autumn) visitor to the Galician Rías. Later data have shown blooms of this species have their epicentre in Aveiro (40.7ºN), Portugal, and information on *D. acuta* developments there constitute the most reliable early warning for later blooms to develop in Galicia (Moita *et al*. 2005; Escalera *et al*. 2010).

DINVER 2006 followed exceptionally late (November) and intense blooms of *D. acuta* and *G. catenatum* the previous year, and the scattered cells of these species detected did not serve our objectives. No overwintering or pre-bloom cells of *D. acuminata* were found in potential retention areas, i.e. anticyclonic eddies identified by the MOHID model predictions in real time, in contrast with findings of "HAB incubators"(of *D. acuminata* in the Bay of Biscay, see Xie *et al.* 2007, and of *Pseudo-nitzschia* spp. in the Juan de Fuca Eddy, NW USA, see Trainer et al. 2009) associated with this kind of hydrodynamic feature. Nevertheless, 2006 was also peculiar concerning its very late initiation of the *D. acuminata* growth season, in late June, following the occurrence of an exceptional bloom of *D. ovum* (Pizarro et al., 2013). The lesson from this cruise is that the absence of winter populations of *D. acuminata* in the Rías Bajas and shelf in early February is a good sign for not expecting early (before June) DSP closures caused by this species. This conclusion was later confirmed in a time series analysis for identification of the causes of phenological changes in the onset of *D. acuminata* blooms (Díaz *et al.* 2013).

DINVER 2013 was carried out four weeks later than DINVER 2006, looking for a time window closer to the initiation of the *D. acuminata* growth season. But 2013 was "different" in having a very early initiation of the upwelling season (favourable for the onset of *D. acuminata* growth, see Díaz *et al*. 2013) and cells of these species were present in the water column throughout the winter. In fact, shellfish harvesting closures due to DSP toxins in the Rias Bajas started in early April. The lesson from this cruise is that detection of *D. acuminata* cells in the Galician Rías and shelf through the winter poses a higher risk of early onset of DSP outbreaks.

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